THE DEVELOPMENT OF THE MICROSPORANGIUM AND MICROSPORES IN CONVALLARIA AND POTAMOGETON.

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(WITH PLATES XXIV–XXV)

DURING the past decade perhaps no one portion of the field of botany has been worked upon so much as that which deals directly with the organs concerned in the sexual process. Especially is this true of the higher plants, but a careful survey of the present condition of our knowledge on this very point shows that some of the most vital questions have as yet received no solution. The researches of such men as Hofmeister, Strasburger, Guignard, and Warming have discovered facts in regard to the ovule, the embryo-sac, and the cell which have already become so universally known as to need no further mention here. But we still know almost nothing about the essential significance of some phenomena of most common occurrence, and many questions have as yet been investigated only in connection with so few plants that generalizations are extremely unsafe. It was principally with the hope of increasing, if only by a few species, the range of observations that the present studies were undertaken.

The choice of subjects signifies very little. It was influenced much more by the necessity of using plants obtainable at certain times rather than by an idea that they all represented different types of structure.

METHODS.

The methods employed in this work differ in no essential way from those so often described in recent cytological works, consequently it is scarcely necessary to repeat them again in detail. The following general statements are intended rather for those who wish simply to know the stains, fixing agents, etc., chosen for the work.

The Flemming chrom-osmo-acetic acid solution has of late years proved of such value that it must now be regarded as the very best fixing agent for cytological work. For the following studies material from no other fixing agent was used, although some was put up in alcohol, sublimate, and picric acid. None of the latter gave satisfactory results. The secret, if there is any, in the use of the Flemming solution seems to lie in obtaining rapid penetration. To accomplish this the material cannot be subdivided too much, and when possible even the anthers themselves should be cut open. From six to twelve hours is sufficient for complete fixation. The black discoloration caused by the osmic acid was removed by the aid of hydrogen peroxid either upon the sections themselves or preferably upon the material *in toto*.

For clearing, cedar-wood oil gave the best results. It was always added with great care in order to avoid too rapid change of density, otherwise collapse of the cells often occurred. The paraffin used possessed a melting point of 54°. This also was added with the same degree of care. The sections ranged in thickness from four to six and two thirds microns, and were all cut on a Minot-Zimmerman revolving microtome.

Considerable experimentation was necessary before a suitable staining method could be devised. Among others, Rosens' fuchsin-methylene blue method,[1] iron-hæmatoxylin and the Flemming safranin-gentian-violet-orange combination were the most important. The latter was at length almost exclusively employed, and was found very satisfactory. As a general nuclear stain, however, better results were obtained by the omission of the safranin, and at the same time a large amount of time was saved. The orange G was always used in a very dilute solution, and for a very short time, from 15 sec. to 2 min. giving the best results. For a chromosome stain the gentian-violet was allowed to act for a short time only, but for differentiating the spindle fibers and kinoplasmic radiations, much better results were obtained with a very weak solution (2–3 drops of the stock

[1] Beiträge zur Kenntniss der Pflanzenzellen. Cohn's Beiträge 5:443. 1892.

solution made according to Lee in 100cc of water) acting for from 12 to 24 hours. After washing out in absolute alcohol, further differentiation was obtained with clove-oil, or in the case of Potamogeton, preferably with anilin oil. The action was then stopped with bergamot oil before mounting in balsam.

CONVALLARIA MAJALIS L.

The Liliaceæ have so far furnished some of the very best subjects for cytological study. To those already studied must now be added another good type, namely Convallaria. This plant has unfortunately up to the present time received very little attention, although the large nuclei and long rod-like chromosomes almost equal Lilium in the ease with which they may be studied. The published observations are at present limited to those of Strasburger on the pollen of *C. Polygonatum*, and on the endosperm of *C. majalis*.[3] The observations on both of these are very brief.

The material for this study was obtained from plants grown in the University greenhouses. They were here under a constant though moderate condition of forcing, which thus enabled one to obtain an unusually large proportion of dividing nuclei. The progressive development of the flowers in the raceme makes it possible to find many stages on a plant all at one time. To insure rapid penetration of the fixing agent the upper and lower ends of each bud were cut away, thus exposing directly the cells of the anther.

In order to determine if there might not be a relation between the nuclear division and the environment of the plant, especially as to the amount of light, humidity, etc., several experiments were made. In the case of Convallaria only the effect of light could be studied, since the other conditions in the greenhouse were practically the same. In order to test the effect of light, material was collected at various times during the day and night. The results, however, were wholly negative. Spindles were found in about the same proportion in every collection.

[2] Befruchtungsvorgänge bei den Phanerogamen 171. 1884.

[3] Theilungsvorgänge der Zellkerne 43. 1882.

THE DEVELOPMENT OF THE MICROSPORANGIUM.

In 1873 Warming's important work on the development of the anther apeared.[4] In this paper we find for the first time a correct description of the succession of cell divisions resulting in the formation of the archesporium and the anther wall. It was not followed up, however, by other investigators, and even in late years nothing of importance has been done along this line. The most important of the subsequent papers is undoubtedly that of Engler in which Orchis is taken up in detail.

Warming established the fact that the archesporium arises from the daughter cells resulting from the division of the hypodermal layer at each corner of the anther. If the hypodermal cells form a true layer, then the archesporium will usually also be in the form of a layer; but in some cases the hypodermal cells may be reduced to one, and the resulting archesporium in that case is simply a vertical row of cells. In any event, there is almost no subsequent division in the archesporium, growth being confined entirely to an increase in size of the existing cells. Of the two original daughter cells of any hypodermal cell, the inner gives rise to the archesporium, the outer to the anther wall. By two or three periclinal divisions progressing in a centrifugal manner a radial row of cells is formed, the inner cell of which becomes the tapetum, the outer the endothecial layer, and the rest finally disintegrate. Although many of the divisions are periclinal, some are radial and others transverse, by which means accommodation is provided for the growth of the archesporium. He finds also that cell division in the epidermis is almost entirely radial and transverse, and this structure remains always one-cell thick. His subjects for investigation, however, included only one monocotyledon; and Engler,[5] who attempted to show more clearly the application of Warming's laws to the monocotyledons,

[4] Untersuchungen über pollenbildende Phyllome und Caulome. Hanstein's Bot. Abh. 2: 1. 1873.

[5] Beiträge zur Kenntniss der Antherenbildung. Pringsh. Jahrb. f. wiss. Bot. 10: 275. 1876.

used Orchis as the only type. At present, therefore, our knowledge of the details in this group is very meager.[6]

In Convallaria the early stages in the development of the anther are not so easily understood as are those of the dicotyledons; but after the investigation of a large number of preparations it seems probable that the following is the proper interpretation, not alone for Convallaria but also for many other monocotyledons. The earliest stages obtained show in cross-section a four-angled anther with a radial row of cells at each angle, which apparently result from the division of a single hypodermal cell.

The condition at this stage is represented in *fig. 1*. The innermost cell, the primary archesporial cell, very soon divides in various directions until a considerable mass of tissue is formed. This division takes place very early, so that the final number of archesporial cells is formed even before the anther has become obviously lobed. At this stage it is only with considerable difficulty that the archesporium can be distinguished from the wall (*fig. 2*). The original radial row of cells, descendants of the primary hypodermal cell, may often be recognized for a considerable time after they are first formed, but in some cases one or two cells on either side may also divide several times in a radial direction. The greater portion of the wall, however, is derived from a few irregular divisions of the cells at each side of the archesporium, while the epidermis is at the same time increased by a few anticlinal divisions. In addition to this the cells between the archesporium and the connective may also undergo a few radial divisions. As a result the wall-cells in the older anthers do not stand in distinct radial rows as in the dicotyledons, simply because they were not all derived by radial division; but, notwithstanding this, there are quite

[6] While in press the following papers have appeared:

GUIGNARD, L.— Le développement du pollen et la réduction dans le *Naias major*. Arch. d'anat. Microscop. **2**: 455. 1899.

CALDWELL, O.— Life history of *Lemna minor*. BOT. GAZ. **27**: 37. 1899.

FULLMER, E. L.— The development of the microsporangia and microspores of *Hemerocallis fulva*. BOT. GAZ. **28**: 81. 1899.

regularly three or four layers of cells differentiated entirely around the sporogenous tissue. These stages were found in the autumn previous to the time of flowering, and all subsequent growth, both in the archesporium and in the wall, is due entirely to the increase in diameter of the cells already formed. This was determined by an actual count of the cells in a great many cases.

In most dicotyledonous anthers the archesporium becomes distinct at a very early period. No such sharp demarcation exists, however, in the monocotyledons. In all of the cases studied by the writer, and in those treated by Warming and Engler, the transition from the wall to the archesporium is so gradual, especially in the younger conditions, that only the most careful study enables one to distinguish accurately between them. Although it is difficult to distinguish them solely by their form, it is not so difficult when their cytoplasm is considered. In Convallaria the cytoplasm of the archesporial cells becomes so modified that by its structure alone one can recognize the future mother-cells. The change consists in an increase in the abundance of the cytoplasm, as well as in the formation of a much finer network with scarcely any indication of a granular structure. Taking this into consideration, the wall is now readily seen to consist of four layers.

From the above description it will be seen that there really is here a special case of Warming's law as formulated for the dicotyledons, and not an entirely new process. In other words, the archesporium seems to arise entirely from the division of one or two hypodermal cells rather than from a layer of such cells, and the wall is formed mostly from cells adjacent to the archesporium.

At a slightly later period the fourth or innermost layer of the wall begins to enlarge. The cells grow considerably, and soon might be mistaken for archesporial cells if it was not for the difference in the cytoplasm. This layer is the tapetum (*fig. 3*).

The history of the tapetal nucleus has been studied by

Strasburger in Malva, but not in detail.[7] In this plant two nuclei were found in each cell. Guignard also figured two nuclei in the tapetal cells of Lilium, and it now seems probable that the phenomenon is quite general, as Strasburger in the above cited work states. It was originally believed that these two nuclei were formed by direct division; but Strasburger[8] has shown that this was really a process of fusion, and that the two nuclei were formed at an earlier date by the ordinary indirect method.

In Convallaria, at the period just preceding synapsis, the already enlarged tapetal cells contain only one nucleus; but during synapsis, and even up to the first pollen-mother-cell division, the nuclei one by one divide by the mitotic method. Such spindles are seen in *fig. 4*. It is probable that every tapetal nucleus finally undergoes division, but this could not be accurately determined because of the subsequent fusion. The different stages in the fusion of these nuclei were sufficiently frequent after the first division of the pollen-mother cell (*fig. 5*). Cases could often be found even in anthers where tapetal spindles also occurred. It is doubtful if all the pairs of nuclei fuse —in fact, it is probable that they do not, since many remain distinct even after the tapetum shows signs of disintegration.

The wall of the mature anther in Convallaria presents no new features. It is composed of a conspicuous epidermis, a well-developed fibrous layer (endothecium) with beautiful spiral markings, and the remains of the other wall layer (*fig. 6*). No remnant of the tapetum is now left. The time of disappearance of this structure was interesting because it occurred in some cases as early as the pollen-mother-cell stage, while in others not until after the pollen grains were mature. The time is seldom the same even in anthers from the same bud.

THE NUCLEUS OF THE ARCHESPORIUM.

The very earliest stages of the archesporial nucleus in Convallaria differ only slightly in appearance from true vegetative nuclei (*fig. 7*). The linin thread is exceedingly thin and fine

[7] Ueber den Bau und das Wachsthum der Zellhäute 89. 1882.

[8] Theilungsvorgänge der Zellkerne 99. 1882.

as well as extensive. Careful examination shows that it is not a continuous ribbon at this period, but rather an anastomosing network in which large granules of chromatin of unequal sizes are imbedded, giving it thus a more or less knotty appearance.

At a period quite early in the development of the anther the chromatin network contracts into a dense and ultimately spherical mass, which in most cases is in contact with the nuclear membrane, but may also lie free in the nuclear cavity (*fig. 8*). The ball becomes so dense that it is ordinarily impossible, even with the weakest stains, to distinguish the separate threads of which it is composed, except at the periphery where they project into the nuclear cavity outside. This condition is synapsis in Convallaria. The nucleolus does not lie inside the mass and adjacent to the membrane, as has been claimed for some other plants. On the contrary, it always lies outside so far as could be determined, but usually in contact with the mass and at the side away from the wall more often than toward it.

Judging from the number of preparations obtained, the nucleus must remain in the synapsis condition for a considerable time. The first indication of the return from synapsis is found in the gradual separation of the outermost linin threads, and finally the whole mass becomes more open (*fig. 9*). But a very strange thing now occurs. At the moment when the chromatic thread is spreading out, between its meshes and in the cavity outside are to be seen large granules, or more properly speaking masses of various sizes which are decidedly chromatic, in fact stain exactly like chromatin. The nucleolus meanwhile still remains intact. Where these bodies come from or where they go could not be determined. They were always present, however, at this stage. The masses were often as large as the nucleolus but more often smaller. It might be suggested that in some way the nucleolus becomes fragmented either naturally or by the action of the reagents, but the preparations do not support this view. In very weakly stained nuclei the nearly colorless nucleolus with its central vacuole could be plainly seen, while in the same nucleus the expelled masses were stained

dark like the chromosomes at the time of division. Again it is possible that the chromatin had left the linin thread and collected in the above manner before the material was fixed. Or perhaps the reagents caused such a change in the chromatin. The investigation of fresh material alone can decide whether the process is normal or artificial.

A remarkable change has also taken place in the chromatin thread. Before synapsis it was a network containing very large irregular granules, now it is a spirem with the granules much reduced in size and more uniform. For the most part they are but slightly broader than the scarcely thickened linin. These above mentioned peculiarities were found not in one case alone, but in a great many preparations, in fact in every preparation that contained the right stage.

Synapsis has now been found in many plants, in all of which it always seems to be a natural condition, and it is quite probable that it will prove to be a universal phenomenon, occurring at a certain period previous to each heterotypic division. Besides this, it has now been shown to occur in at least a few animals. Among plants it has been found in the Liliaceæ by Strasburger, Sargant, and others; in the Hepaticae by Farmer; and in Potamogeton and Acorus by the writer, in addition to several other plants, the studies on which have not yet been published. There now seems to be little doubt that the condition is a natural one for the following reasons. It may be found in the fresh material, at least in Convallaria and Lilium. Moreover, it always occurs at the same stage in the development of the anther. Various structural changes in the chromatin thread always accompany it. In addition to this, many preparations show that the mass may be deposited at any side of the nuclear cavity without reference to its position in the anther.

Considerable uncertainty has always existed as to just what synapsis really is. The term was introduced by Moore[9] in the year 1895, but the description was so brief that it is somewhat

[9] Essential similarity of the chromosome reduction in animals and plants. Annals of Botany 9:435. 1895.

difficult to understand just what the author had in mind. It seems probable, however, that the condition found by him was the same as that described above for Convallaria. He was able to demonstrate the appearance of the same phenomenon also in animals, especially in Triton, thus emphasizing the fact that the steps in the maturation of the sexual gametes is to a certain extent similar in both plants and animals.

During the past few years synapsis has been described by several authors, most of whom now consider it to be a natural condition of the cell, although it seems probable that the phenomenon described is not the same in all cases.

Farmer[10] found a contracted condition of the chromatin thread in the spore-mother-cells of the Hepaticae, but the figures do not show as great a contraction as is found in Convallaria. He states that the nuclei at this stage are difficult to fix, often showing signs of fragmentation, and that there is usually a chromatic change in the cell.

Convallaria has so far shown no case similar to that described by Miss Sargant[11] for Lilium, in which two rows of dots were found on the thread before synapsis. A double row in the former plant is found only very late in the history of the spirem; neither does the nuclear membrane disappear or even become indistinct during synapsis. Otherwise the description and figures are quite similar to Convallaria. In some preparations an extrusion of granules from the contracted mass was found, but was interpreted as a fragmentation of the nucleolus rather than as a separation of portions of the chromatin.

The conflicting results obtained are probably due, in part at least, to the fact lately emphasized by Strasburger,[12] that several distinct phenomena have been referred to synapsis. Only one of these is a normal condition, and to this the term synapsis

[10] On spore formation and nuclear division in the Hepaticae. Annals of Botany 9:482. 1895.

[11] The formation of the sexual nuclei in *Lilium Martagon*. Annals of Botany 10:457. 1896.

[12] Karyokinetische Probleme. Pringsh. Jahrb. f. wiss. bot. 28:158. 1895.

properly belongs. The others are probably caused by the action of the reagents.

Mottier's[13] figures representing synapsis in Lilium do not show nearly as much contraction as in Convallaria. From the description it would seem also that they represent a much later stage in the development of the spirem than the one in which synapsis occurs, either in Convallaria or in Potamageton.

FIRST NUCLEAR DIVISION OF THE MOTHER-CELL.

The spirem stage preceding division is very well marked in Convallaria (*fig. 10*). The much coiled wire-like thread found immediately after synapsis gradually increases in thickness, and the chromatin granules become less prominent. One large nucleolus and usually two or three smaller ones are present at this period. The longitudinal splitting of the chromatin thread is accomplished so quickly that it was found impossible to observe the successive steps in the process. At the same time the thread becomes considerably thicker than immediately before the division (*fig. 11*). It is difficult to understand just how this doubling takes place, because at the very first indication of such a condition the threads are already separated. They lie parallel with each other and slightly coiled. The further development indicated a more or less complete subsequent fusion of the parts, so that before passing into the nuclear-plate stage the dual nature is entirely lost except for an occasional lobing at either end (*fig. 12*). Not even the granules are longer visible, and the chromosomes are at this stage apparently homogeneous. This is probably only apparent, however, and due really to the density of the stain and the close proximity of the parts.

After the chromatin thread has become double, besides being thicker than before, it also possesses fewer coils, which is probably due to a longitudinal contraction of the whole spirem. At about the time when the nuclear membrane disappears the thickened chromatin band segments into the individual chromosomes

[13] Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Pringsh. Jahrb. f. wiss. bot. **30**: 175. 1897.

(*fig. 12*). The successive stages in the process of segmentation could be easily traced. The constrictions gradually become deeper and deeper, while at the same time the chromatin is withdrawn from the constricted regions. The resulting segments were always of the same length as the mature chromosomes appearing on the nuclear plate. Repeated examination of these stages failed to reveal a V-shaped bending back of the segments corresponding to that described by many investigators for Lilium. Moreover, the chromosomes do not have four lobes at the end, as would be more likely the case if they were formed by the folding-back process, but only two. It seems, therefore, that in Convallaria at least the chromosomes, although double in nature and hence sometimes showing a longitudinal split, are always straight and formed simply by the transverse fission of the chromatin thread.

The changes which the chromosomes pass through while on the nuclear plate are very difficult to make out, and consequently little that is definite can be said about them. The segments seem to be straight or slightly curved and lie mostly in a radial manner on the plate (*figs. 14, 19, 20*). The earlier stages show a nearly cylindrical chromosome, but very soon this becomes changed into the characteristic +-like structures which are very commonly seen at this stage. These structures seem to be formed, as Belajeff[14] and Strasburger[15] have shown, by a simultaneous splitting at each end of thc cylinder, but in perpendicular planes. The inner forks are drawn apart by the spindle fibers, while the two outer ones separate in the plane of the nuclear plate. In many cases a fissure may be seen to extend from the apex of one long arm directly through the middle of the chromosome (*fig. 20*).

The two V-shaped segments resulting from the separation of the two halves of the + pass to the poles in the ordinary manner (*figs. 14, 15, 22*). The daughter segments proceed with the

[14] Zur Kenntniss der Karyokinese bei den Pflanzen. Flora, Erganzungsb. **79**: 434. 1894.

[15] Karyokinetische Probleme. Pringsh. Jahrb. f. wiss. Bot. **28**: 183. 1895.

angle of the V in front, as one might expect if the spindle fibers are assumed to be exerting a pull upon them. They are always more slender than the parent chromosomes, which would be the case if a division of the original substance had taken place. Occasionally a V will straighten out and lie along the spindle fiber, reaching almost from the equator to the pole.

The spindle in Convallaria is formed in the same way as in Lilium. The disappearance of the membrane is exactly coincident with the appearance of the kinoplasmic threads which immediately penetrate the nuclear cavity, and also extend outward into the cytoplasm (*fig. 12*). Very little light, however, could be thrown on the fate of the nucleolus, and its disappearance was very sudden. The multipolar spindle is not very distinct in this plant (*fig. 13*), and the poles scarcely ever extend much beyond the limits of the old nuclear membrane, and are often difficult to distinguish at all. The bipolar spindle is usually truncate at the poles, but unlike that of Potamogeton, it is broad and barrel-shaped, possibly due to the large number of chromosomes (*fig. 14*). In this respect it is similar to Lilium. In no case was there even so much as a granule present at the pole, or any thing that could be mistaken for a centrosphere. A strong nuclear plate follows the division, resulting in a cross wall separating the cell into two hemispherical parts.

SECOND NUCLEAR DIVISION OF THE MOTHER-CELL.

The resting stage between the first and second divisions in Convallaria is very short. One may often find the nuclei at one end of an anther in the cell-plate stage, while those at the other end have formed the nuclear plate for the second division. The daughter nuclei do not seem to pass entirely into a normal resting condition. So far as could be determined, no nucleolus ever appears, nor does the nuclear membrane become well developed, however a very delicate membrane may often be observed. The chromosomes apparently retain their identity throughout this stage. From the pole view it can be determined that they still possess their V-shaped form (*fig.* 16).

The transition from the resting stage to the second spindle is very abrupt. Two poles are formed in the cytoplasm, which move farther apart, so that a bipolar spindle is very quickly formed (*fig. 17*). In the preparations examined, no polar radiations or multipolar spindles were found. The already distinct chromosomes merely move together toward the center in order to form the nuclear plate. Even now most of them retain the V form, and only an occasional one becomes nearly straight. They are very irregularly arranged, so that the long arms of some project outward toward the poles, giving a ragged appearance to the plate, thus distinguishing it immediately from the plate formed in the heterotypic division.

It was found impossible to determine absolutely the nature of the segmentation, but from a study of all the stages obtainable it seems probable that the V is divided transversely. This of course would not mean a transverse division of the original chromosome, if the processes described for the first division are the true ones, but rather the completion of a second longitudinal splitting. No figures were found in any of the spindles that could be interpreted as a case of undoubted longitudinal splitting of the V after coming on to the nuclear plate of the second spindle. On the other hand, the nearly straight segments moving along the spindle toward the poles are all much shorter than the Vs, but of approximately the same diameter instead of narrower, as one would expect if longitudinal splitting had taken place.

The later stages are all perfectly normal. The chromosomes arrange themselves in the daughter nuclei and appear at length to fuse into a continuous chromatin thread. The nuclear membrane does not appear at once, but by the time the young pollen grains are differentiated it is usually evident.

The spindle during the second division, both before and after the passage of the segments to the poles, is much less distinct than during the first division, and it is composed of fewer fibers. After the daughter nuclei are formed, a cell-plate is deposited in the usual manner. The spindle now disappears and the tetrad division of the pollen-mother-cell nucleus is complete.

The number of chromosomes in Convallaria is quite large. A count in the nuclear plate stage showed eighteen segments as the reduced number. The same number may be counted during the subsequent resting stage and also after the second division.

THE MICROSPORES.

After the second division of the mother-cell nucleus the young pollen grains do not separate immediately, but remain a short time inclosed in the thickened walls of the parent cells. With little difficulty one can follow all the steps in the process of dissolution which these walls undergo. First the increasing sponginess of the already thick wall; a simultaneous differentiation of its inner layer destined to become the wall of the spore; and finally the complete solution of the outer part, leaving the young pollen grains united only by the intervening walls. These apparently split at once into two layers, thus freeing the members of the tetrad.

The pollen grains at first are quite small, and possess thin purple-staining walls, finely granular cytoplasm similar in consistency to that of the somatic cells, and a highly chromatic nucleus which occupies about one fourth of the cell-cavity (*fig. 23*). The further changes are mostly normal. The pollen grains, which from the first are elliptical, gradually increase in size until their volume is more than doubled. The wall increases in thickness, and the whole grain assumes a bluer tinge with gentian-violet.

A short time before the flower opens, the nucleus undergoes division, whereby a generative cell is cut off (*fig. 24*). This cell is lenticular in form, and separated from the general cavity of the grain by a distinct cell wall. The generative nucleus is exceedingly chromatic, so much so in fact that it stains almost a homogeneous dark purple with the gentian-violet. The generative cell in Convallaria seems to differ from those in most of the monocotyledons described by other writers in not separating at an early period from the wall of the pollen-grain. It apparently remains in all cases attached until the time of pollination.

The division of the generative nucleus into the two sperm nuclei must take place in the pollen tube, good stages of which were not obtained. Except in the one point above mentioned, the microspores of Convallaria do not differ in any essential way from those described by Strasburger[16] for a large number of the higher monocotyledons.

POTAMOGETON FOLIOSUS RAF.

The numerous investigations recently made upon plants belonging to the orders Alismaceæ and Naidaceæ have shown that many peculiar conditions are to be found among these groups of monocotyledons. During the past summer the writer was able to procure excellent material of *Potamogeton foliosus* in the ponds about Ithaca ; and it was decided to make a detailed study of this plant for comparison with the studies already made by others. No one seems to have investigated this genus from a cytological standpoint.

Of the papers on nearly related plants must be mentioned that on Naias by Magnus,[17] on Naias and Zannichellia by Campbell,[18] and on Alisma[19] and Sagittaria[20] by Schaffner.

The material was collected during the months of July and August, at which time the oldest flowers are just producing fruit. The floral spikes mature in succession as the plant branches, so that the very youngest flowers and also the fruits may be found upon the same individual. There being no cutinized layer surrounding the bud, the latter is especially easy to penetrate with the fixing agent. The material used for this study was therefore in exceptionally good condition. As in the case of Convallaria, the collections were made at certain times during the day and night, and the result also was exactly the same.

[16] Befruchtungsvorgänge bei den Phanerogamen 22. 1884.

[17] Beiträge zur Kenntniss der Gattung Naias. Berlin. 1870.

[18] A morphological study of Naias and Zannichellia. Proc. Calif. Acad. Sci. III. 1:1. 1897.

[19] The embryo sac of *Alisma plantago*. BOT. GAZ. 21:123. 1896.

[20] A contribution to the life history of *Sagittaria variabilis*. BOT. GAZ. 23:252. 1897.

THE DEVELOPMENT OF THE MICROSPORANGIUM.

To trace the development of the anther, and especially the differentiation of the microsporangial archesporium in Potamogeton, is no less difficult than in Convallaria. Oddly enough, the only monocotyledonous type studied by Warming was one of the Naiadaceæ, namely Zannichellia. Warming thought that in this plant the process was essentially identical with that in the dicotyledons, but these results may be questioned, owing to the apparent paucity of material at his command.

The present study of Potamogeton seems to throw a little more light on the problem. In the young anther, which at maturity is always two-celled, there is found at each of the two more prominent angles of the quadrangular cross-section a single hypodermal cell, which at this stage is slightly larger than the surrounding cells and richer in protoplasm. This presently is divided by periclinal walls into two, and later into three daughter cells, each produced probably in centrifugal succession (*fig. 25*). The innermost of this series now immediately begins to enlarge, and becomes at once the primary archesporial cell. This cell undergoes rapid division, resulting at length in a number of cells, all formed from this one original archesporial cell. The irregularity in arrangement, and the gradual decrease in size from the center toward all sides, nevertheless suggest that some may owe their origin to the division of the surrounding tissue. The same gradual decrease in size takes place also on the side of the archesporium toward the connective.

It will be seen from the above account that the tapetum here and in Convallaria is not a morphologically distinct structure until at a comparatively late period in the development of the anther. It is not until the stamen is half mature that the archesporium becomes distinct from the wall. It can always be recognized at this period by the finely granular contents of the cells, just as was the case in Convallaria. No tapetum can be distinguished for some time. The cells of the inner layer of the wall, which from the first are smaller than the central cells, gradually take on a dense and partially disorganized appearance. There is

no longer any doubt but that the tapetum is differentiated from the wall, rather than from the archesporium, as a hasty inspection would seem to indicate.

The wall of the anther at this stage is composed of three, or rarely four, layers of cells (*fig. 26*). The outermost of these layers remains almost unchanged until the anther is mature, and is indeed the true epidermis. The other two or three layers have some of their cells arranged in more or less distinct radial rows, suggesting, as in the first case, that each row is the derivative of one hypodermal cell. The greater portion of the wall, however, is formed, as in Convallaria, from the cells of either side of the archesporium, and these are not necessarily derivatives of a hypodermal cell. Indeed, so far as could be determined, the growth was brought about exactly as in Convallaria. During the maturation of the anther the behavior of the cells is normal. The third layer undergoes disintegration, as does also the fourth, which is the tapetum. The epidermis remains normal, while at the same time the second layer becomes thicker walled than the rest, acquires spiral or reticulated thickenings, and is indeed a true endothecial layer (*fig. 27*).

Campbell considers the anther of Naias to be a so-called "caulome" structure, in which are early differentiated plerome and periblem, the upper cell of the plerome cylinder becoming the archesporium. This in itself does not preclude a process similar to that described above for Potamogeton; although Campbell himself is quite certain that the origin of the archesporium in Naias is not traceable to a single cell. He found the wall composed of only two layers besides the epidermis, instead of three. In Zannichellia the same difficulty was found in tracing the development, but this was probably because the tapetum was not counted as a wall layer. The archesporial cells are here also at first scarcely distinguishable from the adjacent cells. In this plant there are three layers surrounding the archesporium, all of which finally become disintegrated.

The complete disintegration of the tapetal cells in Potamogeton is almost coincident with the divisions of the pollen-mother-cell.

When the young pollen grains are free in the anther, therefore, only a disorganized mass of protoplasm is in the position formerly occupied by the tapetum. This substance is very soon distributed among the pollen grains, where it possibly serves as nutriment. The tapetal cells of Potamogeton never contain two nuclei. In this respect, therefore, they differ decidedly from Convallaria.

THE ARCHESPORIUM AND MOTHER-CELLS.

Division in the primitive archesporium ceases at an early period, after which the development is confined to growth and constitutional changes in the cells already formed. The definitive archesporial cells are at first quite small, but during the long period of growth that now commences they double or even triple their original size. The mature pollen-mother-cell contains a very large nucleus surrounded by abundant cytoplasm. Unlike most monocotyledons, the cell wall here remains very thin, and does not become irregularly thickened, as in Convallaria and Lilium (*fig. 35*). A similar condition has been observed also in Naias and Zannichellia. A very short time, therefore, is required for disintegration, which undoubtedly accounts for the almost immediate separation of the young pollen grains.

THE ARCHESPORIAL NUCLEUS.

Potamogeton belongs to an entirely different class from Convallaria so far as the nuclei are concerned. The Lilium type, to which the latter plant belongs, possesses the well-known dense spirem and the large oblong chromosomes. The nuclei of Potamogeton are apparently very poor in chromatin. The few chromosomes are small and spherical and the spirem very meager. A detailed comparison with Convallaria, therefore, will be especially interesting.

The very young archesporial nuclei in Potamogeton are scarcely different from the surrounding vegetative nuclei. They are surrounded by a definite membrane, have a large nucleolar-like body, and a very poor linin network, which lies close to the

wall (*fig. 28*). As the cell gradually expands, the nucleus also increases in size. The nucleus just before synapsis has already acquired nearly its full size, and the linin network composed of very slender threads is plainly visible. In it are irregularly distributed a few large and small granules of chromatin. Both the linin and the granules are exceedingly meager as compared with Convallaria. The nucleolus is a gigantic body, much larger than those in most plants, and takes the gentian-violet stain readily, making it thus a very striking object in the cell. Attached to it on one side is a small wart-like body only slightly larger than the largest chromatin granules. Rarely two of these are present. This body appears very much like a bud produced by the nucleolus itself, but in reality is not. If specimens are examined which have been poorly stained, in which the gentian-violet has been mostly washed out, this little body remains dark much longer than the nucleolus. A fine double stain can often be thus obtained. The nucleolus takes the orange in that case, while the other body stains violet. From the fact that this larger body always stains like ordinary chromatin, and since there is scarcely any chromatin upon the linin thread, the writer is inclined to believe that it is not the nucleolus, but a large mass of chromatin similar to those found in many animal cells. The nucleolus is possibly the wart-like body attached at its side. The behavior of the larger body when the chromosomes are being formed seems also to support this view.

The synapsis stage in Potamogeton is even more marked than in Convallaria (*fig. 29*). The linin network contracts into a globular mass lying in contact with the nuclear wall, but not noticeably pressed against it. With the highest magnification, the central part of the mass still appears too dense to distinguish any structural characters. On the periphery, however, the free ends of the network may be seen easily. The large nucleolus, accompanied by the wart-like body, remains in its central position in the nucleus throughout the synapsis stage. It always stains more deeply than the linin. The mass of linin, therefore, lies between the nucleolus and the nuclear membrane. The later

stages of synapsis are marked by the same striking peculiarities that were met with in Convallaria (*fig. 30*). In just the same way large globules of some deeply stainable matter accumulate on the outside of the contracted mass, appearing as if expelled from it. They seem, however, to be distinct from the nucleolus, as there is no apparent fragmentation nor budding of the latter. The nucleolus throughout the whole process remains of exactly the same size and regular contour, and with the little wart-like attachment undisturbed.

The spirem stage is much shorter than in Convallaria. The contracted linin network gradually begins to expand until the threads are again spread out beneath the membrane. Here again we notice a decided change in the structure of the linin thread, just as was the case in Convallaria. It is no longer so slender, and provided with such large granules, nor is it so conspicuously in the form of a network. The spirem is composed of a few rather thick linin threads extending in various directions around the nucleolus, and crossing each other occasionally. In them appear small chromatin granules, which, however, are much smaller and more regular in size than those present before synapsis (*fig. 31*). The whole process, therefore, is exactly comparable with that in Convallaria.

FIRST NUCLEAR DIVISION OF THE MOTHER-CELL.

The stages in the nuclear development preparatory to the first nuclear division are not nearly so marked as in Convallaria. The first indication of division is found in the gradual massing together of the chromatin into a number of irregular masses simulating those found just before synapsis. The difference lies in their larger and more equal size. Just at the time when the nuclear membrane is disappearing the number of these masses of chromatin may be determined approximately. Probably fourteen or sixteen is the correct number. They soon seem to lie together in pairs, in which case two may be easily mistaken for one (*fig. 32*). During the later stages the two parts of each pair seem to lose their identity, so that when on the spindle

it is not possible to count more than seven or eight chromosomes.

Whether a fusion takes place here one cannot determine, since the small size renders it impossible to follow the process accurately. The exact manner of segmentation upon the spindle is also still in doubt. Seven chromosomes are found in the daughter nuclei, which leads us to infer that each of the original masses splits into two, but the preparations show no indications of any such division farther than that in many cases under high magnification, it seemed as if the chromosomes possessed a +-like structure similar to that in Convallaria, but the figures were not distinct enough to allow of any definite conclusions.

In regard to the formation of the spindle a few notes may be given, although the results do not differ essentially from those obtained by Mottier in Lilium. The kinoplasm is at first limited to a thin felt-like coat surrounding the nucleus (*fig. 32*). On account of the very large space occupied by the nuclear sap, it is easy to observe the entrance of the kinoplasm into the nuclear cavity. This takes place apparently before the entire disappearance of the membrane. The latter sometimes is still visible after the nuclear cavity is nearly filled with kinoplasm. At first thought it seems impossible to conceive of a substance passing through the nuclear membrane in this way. But Mottier[21] has shown that the membrane itself is probably nothing more than a close weft of kinoplasm. We have then merely to assume that the inner threads of this weft separate from the rest and traverse the nuclear cavity instead. Finally the whole membrane is entirely transformed into radiating threads. Thus it is not necessary to conceive of the kinoplasmic threads penetrating the membrane. They are from the first a part of it. At first the spindle is multipolar (*figs. 33, 34*), but the poles are few in number and very soon disappear, thus giving place to the normal bipolar type. In its mature condition the spindle is narrow and the poles are very acute (*fig. 35*). The fibers

[21] *Op. cit.* Pringsh. Jahrb. f. wiss. bot. **30**: 176. 1897.

are few in number, probably not exceeding the number of chromosomes.

In many cases the point at the pole toward which the spindle fibers converge was occupied by a granule both in the first and second division spindles. This granule in well-stained preparations was always dark, but its inconstant occurrence was decidedly against its being considered a permanent structure. The cell plate forms before the spindles of the second division (*fig. 36*).

The body, which looks so much like a nucleolus, disappears previous to the first division at almost the same time as does the nucleolar membrane. At this period it presents a more or less irregular and lobed appearance, but vanishes so quickly that it was impossible to determine whether the process was one of fragmentation or solution.

SECOND NUCLEAR DIVISION OF THE MOTHER-CELL.

Before the second division there is a distinct resting stage. An indistinct membrane is formed, and even a nucleolar body may appear. This latter, however, never becomes so large as in the archesporial nucleus, and often seems to be entirely absent, or at least indistinguishable from the chromosomes (*fig. 36*). A thick linin thread is usually formed, but the chromosomes remain distinct. In this character Potomageton agrees well with Convallaria. During this resting stage it is again possible to count the chromosomes, when the number is still found to be seven or eight. This resting nucleus can be distinguished easily by its much larger size from the one formed after the second division.

The origin of the spindle could not be traced, but many preparations showed it in the mature condition (*fig. 37*). These spindles are smaller and more slender than those described above. Like the latter, they have very pointed poles, and in both cases the poles are almost if not quite in contact with the cell wall.

The chromosomes are closely aggregated in the nuclear

plate stage, so that it was impossible to determine just what happened to them at this point. The daughter chromosomes move to the poles very evenly, that is, with the same degree of rapidity (*fig. 38*). A count here showed the seven segments present. Although the daughter nuclei are quite small, the chromosomes remain distinct for some time, and can be again counted. After a time the nucleolar bodies also reappear, and the division is then complete.

THE MICROSPORE.

Our knowledge of the internal structure of the pollen grain really dates from the time of Hartig.[22] In this author's work is the first mention of the discovery in Tradescantia and several other plants of two nuclei in the pollen. This important discovery seems not to have been noticed by subsequent investigators until Strasburger's exhaustive work appeared in 1877.[23] It was not until then that the fact was generally recognized that two nuclei are to be found sooner or later in the development of every angiospermous microspore. This author was also able to demonstrate that the larger of these two nuclei is to be considered as a vegetative or prothallial nucleus and the smaller a generative nucleus.[24] Strasburger found also that a second division takes place regularly in angiosperms, either in the spore itself or in the pollen tube just before fertilization. Two sperm cells are thus formed from the one generative cell.

Since that time many investigators working upon widely different plants have found two nuclei, and these observations all lead to one result, namely the confirmation of Strasburger's observations in every essential particular. The time of formation, ultimate shape of the generative cell, and the time when the latter divides were indeed not always the same in different plants; on the contrary, all degrees of variation were found, some of which are noted below.

[22] Botanische Untersuchungen aus der physiologische Lab. Land. Lehrung. Berlin, herausg. Karsten 3: 294. 1866.

[23] Befruchtung und Zelltheilung 18, 1877.

[24] Befruchtungs Vorgänge bei den Phanerogamen 5, 1884.

The microspores of Potamogeton become separate immediately after the second division of the mother-cell. The anthers are at this time still quite small, the subsequent growth being in reality for the purpose of accommodating the increase in size of the pollen grains. The microspores at first have a thin, although distinct and homogeneous cell wall surrounding the cytoplasm, and a very large nucleus. The latter fills at least one fourth of the whole cavity of the cell (*fig. 40*). The limited amount of cytoplasm present at this time is decidedly much more homogeneous than in the mature pollen, and stains with the gentian-violet a uniform pale violet similar to that of the cell wall. For a short time after the wall of the mother-cell disintegrates the pollen grains are still held together by the remains of these walls. In fact they are as if imbedded in a ground mass of some viscid matter.

The young grains very soon begin to increase in size, but the cytoplasm does not keep pace. As a result, the latter at length is confined to the parietal layer, but with a considerable increase in thickness on the side where the nucleus is located. These stages occur when the embryo-sac is yet one-celled, and of course while the spike of flowers is still enclosed within the bud.

Just before the nucleus begins to prepare for division we find the following conditions: The cytoplasm is decidedly more granular, and stains more deeply with the orange. The large vesicular nucleus possesses a very distinct membrane. Lying close against this is the linin thread which is rather extensive for Potamogeton. The thread however is nearly destitute of chromatin. The nucleolar-like body is smaller than usual, and in some cases more than one may occur.

The first division of the microsporial nucleus takes place much earlier than in Convallaria, and while the whole spike is yet enclosed in the bud. The spores reach their full size before the division, and it is at this time that the exine first begins to show signs of the thickening which produces the very slightly roughened surface of the mature spore. Owing to the small

number of chromosomes present, the spindles in the pollen grain are exceedingly minute and slender (*fig. 41*). The process of division here does not seem to present any new features. When preparing for division, the primary nucleus moves toward one side of the cell, so that the resulting spindle has one pole in contact with the cell wall. This pole unlike the free one, is not pointed; on the contrary, it is usually quite broad, so that the spindle fibers are attached to the wall over a considerable area. The spindle is quite dense and stains readily, but is composed of few fibers. After the chromosomes pass to the poles a distinct cell-plate forms, and is later followed by a definite membrane (*fig. 42*). The latter is arched in such a way as to cut off one daughter nucleus in a small lenticular cell, of which one wall is the wall of the spore itself. This is the so-called generative cell.

The cytoplasm henceforth occupies the greater part of the cell cavity. It gradually becomes filled with large bodies which stain purple with gentian-violet, and blue with iodine. They are in reality starch grains. A similar occurrence of starch in the pollen grain has been described in Naias by Campbell.[25]

After a short period of rest the generative cell begins to elongate, notwithstanding the fact that it still appears to be closely attached to the wall. The elongation produces an oblong cell, and is the first step in preparation for the second division. The chromosomes for this division are formed early. They can often be seen to occupy nearly the entire nuclear cavity, and so distinct are they in many cases that one may count them. The number here again is uniformly seven. The spindles were found in considerable numbers, one of which is seen in *fig. 43*. The spindle fibers are very coarse and apparently scarcely more numerous than the chromosomes. They seem to stain more easily than is ordinarily the case in this plant. The chromosomes are during this division exceedingly minute.

[25] Proc. Calif. Acad. Sci. III. 1: 16. 1897.

The cell plate is soon deposited, and divides the generative cell into two parts. The two daughter cells do not separate, but remain connected as a two-celled body during their entire stay in the spore. A more or less prominent constriction often occurs at the middle, but this does not seem to be constant (*fig. 44*). The pollen grain is always somewhat flattened, and since the generative nucleus is usually adjacent to the flat side, it is not possible to tell whether the latter remains attached to the wall after division. The difficulty was increased since the pollen escapes from the anther very soon after the second division.

An examination of the literature relating to the pollen grain of the monocotyledons furnishes some interesting facts. Strasburger found the division of the generative cell to take place within the spore only in Juncus and Arum; while in all other cases the division was in the tube.[26] All these cases belong either to the Liliaceæ, Orchidaceæ, Amaryllidaceæ, or Iridaceæ. Schaffner found the division occurring in the spores of Typha, Alisma, and Sagittaria,[27] and Campbell found the same to be the case in Naias, while the writer finds the same phenomena in Acorus and Potamogeton. In all cases among the monocotyledons, where division occurs in the pollen grain, with the exception of Alisma and Sagittaria, the generative cell is at first enclosed by a wall, and always becomes two-celled after division, although Campbell claims that the two cells in Naias separate before passing into the tube. Schaffner was not able to discover any walls around the generative cell in the two above mentioned species. From this it appears that the division of the generative nucleus in the tube is mostly confined to the liliaceous and orchidaceous groups among the monocotyledons, while the division within the spore characterizes the spadiceous and naiadaceous groups.

[26] Befruchtungsvorgänge bei den Phanerogamen 22. 1884.

[27] A contribution to the life history of *Sagittaria variabilis*. BOT. GAZ. 23:252. 1897.

SUMMARY.

The following brief summary may aid in bringing together the results reached in the foregoing pages.

The experiments with regard to the effect of external conditions on nuclear division both in Convallaria and Potamogeton gave no results for light and humidity, which were the only conditions tested.

The material illustrating the younger stages in the development of the microsporangium shows that the process is slightly different in Convallaria and Potamogeton from the normal method as given by Warming and Engler. The archesporial cells arise by the division of a hypodermal cell at one corner of the anther. Therefore, instead of the archesporium arising from a layer of hypodermal cells, as Warming describes for dicotyledons, it arises from one or rarely two hypodermal cells. The primary archesporial cells divide only a few times, but there is considerable subsequent growth in size of each cell. The next outer cell in the original row forms part of the tapetum, and the remainder are wall cells. Most of the wall and tapetum, however, is formed from the tissue at either side of the archesporium and in its rear. This differs from Warming's views mainly in the restriction of the hypodermal cell and in the derivation of the wall from the adjacent tissue. The anthers of all other monocotyledons which the writer has had an opportunity to examine seem to show that this is more likely the normal process for the whole group.

The tapetal nuclei of Convallaria show nicely the process of nuclear fusion which has been described by Strasburger and others for many other plants. After the division of the primary tapetal nucleus by the mitotic method, the two daughter nuclei in many cases fuse again, and all stages of the process may be found often in the same anther. It is probable that not all the nuclei divide, and also that not all of those that do divide fuse again before disintegration. It seems that in Potamogeton no division of the tapetal nuclei takes place.

The structure of the wall in the mature microsporangium

was found to agree with the monocotyledonous type in general. The sequence in development was centrifugal and resulted in a well-defined endothecial layer, together with two or three inner wall layers in addition to the epidermis. At maturity only the epidermis and endothecial layer are present.

The development of the archesporial nucleus shows some very important features. The contracted condition called synapsis is without doubt a normal process accompanied by radical changes in the chromatin thread. The latter, which before synapsis was in the form of a network in which were imbedded large irregular chromatin masses, after synapsis is thicker, coil-like, and with the chromatin in smaller more equal masses. The spirem therefore begins at the close of the synapsis stage. In both plants studied irregular dark masses were apparently expelled from the chromatin thread at the close of the synapsis, and in Potamogeton at least it was plainly evident that these had no connection whatever with the nucleolus. The ultimate fate of this chromatin-like matter was not determined. Whether this phenomenon was artificial or natural could not be determined from the material at hand.

The growth and segmentation of the spirem in Convallaria is almost identical with that in Lilium as described by Mottier. The longitudinal splitting of the ribbon is especially noticeable. In Potamogeton the process is different but could not be worked out satisfactorily owing to the minute size of the nuclei. In this plant sixteen chromatin masses were counted just before division, but later there were only about seven, seeming to indicate a fusion of the widely separated primary segments to form the chromosomes. The number of chromosomes after reduction was eighteen in Convallaria and seven in Potamogeton. This last number is one of the smallest so far recorded for the phanerogams.

Spindle formation in all three plants agrees in every essential particular with the process described by Strasburger and Mottier for Lilium. The multipolar condition was evident in each case, but was less distinct in Convallaria.

The splitting of the chromosomes in the heterotypic division

was in Convallaria exactly similar to that in Lilium. All the stages were especially clear. The + formation, however, began in some cases during the early multipolar condition. The process in Potamogeton was probably also normal.

Nothing new could be determined in regard to the segmentation during the second division. It seemed to be absolutely impossible to determine in these plants with any degree of certainty whether the division was transverse or longitudinal. All the phenomena, however, seemed to indicate a transverse rather than a longitudinal division in both plants.

The walls of the mother-cells in Potamogeton were thin as in Naias and Zannichellia.

In the microspores of Convallaria the generative nucleus is very chromatic, and is cut off by a distinct wall, but does not become detached from the wall of the spore until just previous to the time of passing into the tube. The division of the nucleus is probably in the tube since it was not found within the spore. As in Convallaria, so also in Potamogeton the generative cell is cut off very early, but in the latter plant the two sperm cells are immediately formed. The two male nuclei are inclosed each within its own cell wall, but they both still remain attached to the wall of the spore. The two-celled body then passes down the tube and even into the egg without separation of the two cells. The spindles in each case are very small and the chromosomes very minute.

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EXPLANATION OF PLATES XXIV–XXV.

PLATE XXIV. Convallaria majalis L.

FIG. 1. One of the angles of a young anther in cross-section, showing the row of cells derived from the primary hypodermal cell; the two inner are doubtless archesporial cells; the third will form part of the tapetum.

FIG. 2. An anther cell at a later stage; the two archesporial cells in *fig. 1* have divided several times forming a small mass of tissue; the row of wall cells at the top is probably equivalent to that in *fig. 1*; the tapetum is not yet formed.

FIG. 3. A section of the anther wall at a still later stage; on the extreme outside is the epidermis; then two layers the inner one of which will later disintegrate; the fourth layer is the tapetum the cells of which often contain two nuclei; and farther inside are shown a few archesporial cells.

FIG. 4. A portion of the anther wall at nearly the same stage as in *fig. 3*, showing the division of the tapetal nucleus.

FIG. 5. A slightly later stage of the same in which the daughter nuclei formed in *fig. 4* are in the process of fusion.

FIG. 6. A portion of the mature anther wall; the outer layer is the epidermis, the next below is the endothecium with spiral markings on the walls; and farther inside a disorganized mass composed of the tapetum and the one or two layers just outside.

FIG. 7. An archesporial nucleus in the resting stage; the linin network contains granules of chromatin of various sizes.

FIG. 8. Synapsis, the projection at the left is the nucleolus.

FIG. 9. Last stage of synapsis; the spirem ribbon is opening out, and between its meshes are chromatin masses of various sizes; the nucleolus at the left.

FIG. 10. The spirem ribbon with chromatin granules imbedded in the linin.

FIG. 11. The spirem ribbon after longitudinal segmentation, and cut in lengths by the section knife.

FIG. 12. Chromosomes still showing the double nature; dissolution of the nuclear membrane.

FIG. 13. The multipolar spindle with chromosomes in various views.

FIG. 14. The bipolar spindle and nuclear plate; the chromosomes from the end appear +-shaped, from the side view more elongated.

FIG. 15. The + separates into V-shaped segments which are seen moving toward the pole.

FIG. 16. During the resting stage before the second division, the nucleus viewed from the pole; the chromosomes remain distinct.

FIG. 17. The nuclear plate of the reducing division; the segments are so numerous that their form can be determined only with difficulty.

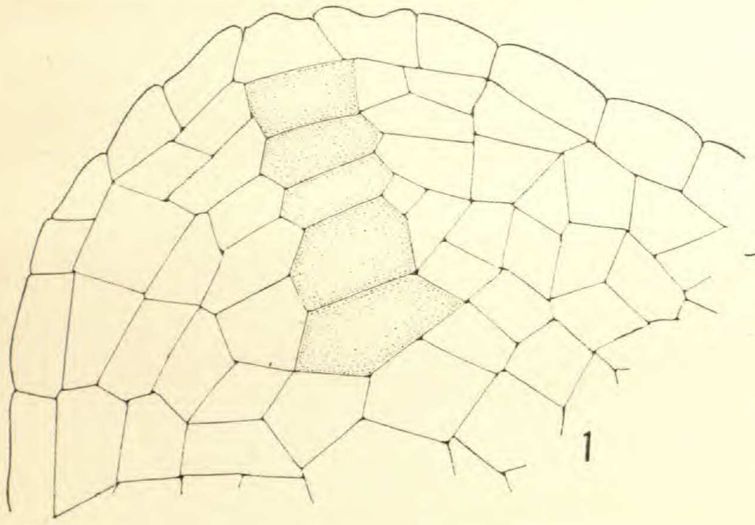
FIG. 18. A chromosome on the nuclear plate, side view.

FIG. 19. Pole view of a chromosome which is bent V-shaped.

FIG. 20. End view of *fig. 18*.

FIG. 21. A rare case, where *fig. 20* has opened out along the fissure line forming a ring.

FIG. 22. V-shaped segments ready to pass to the poles.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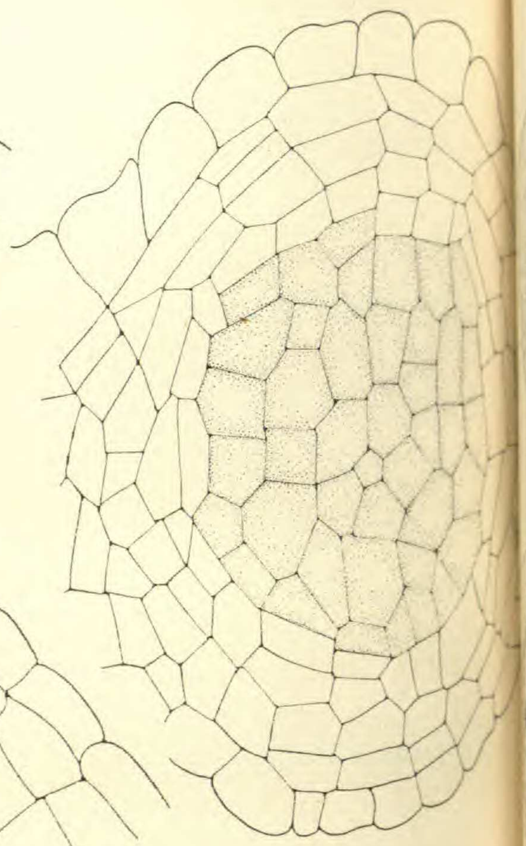
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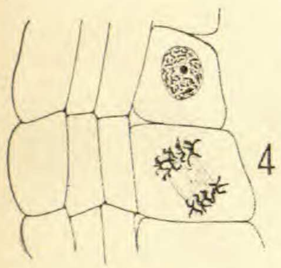
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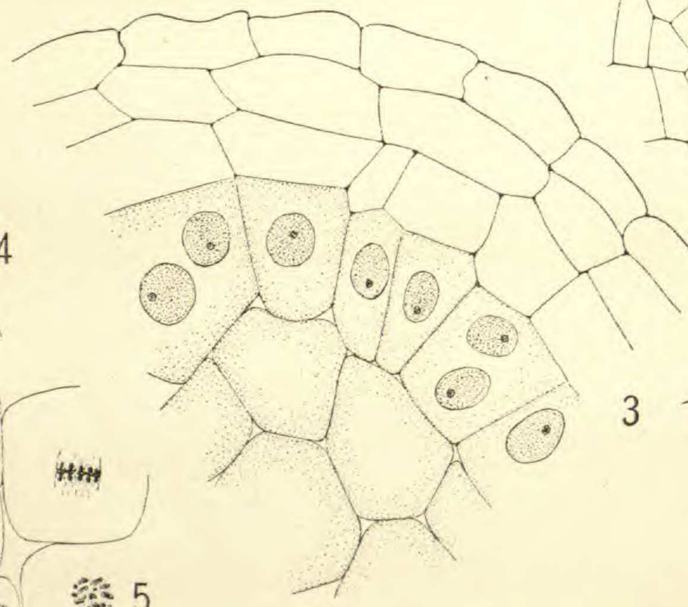
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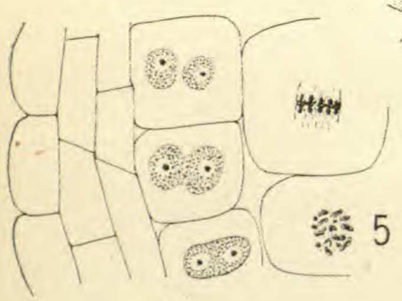
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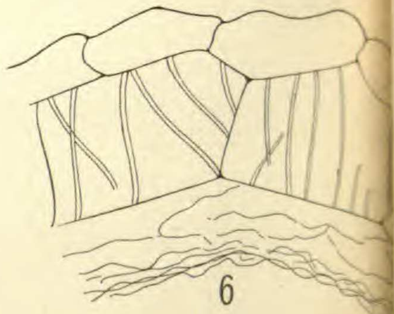
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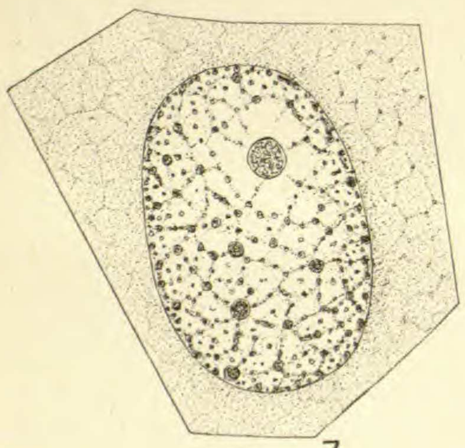
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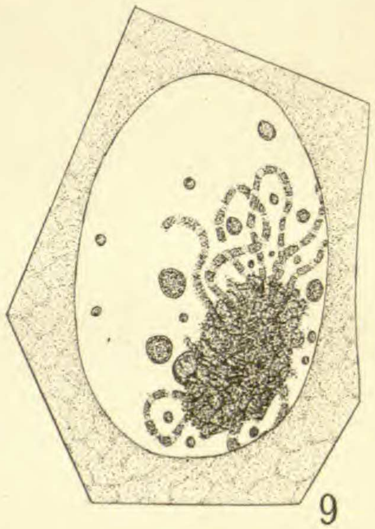
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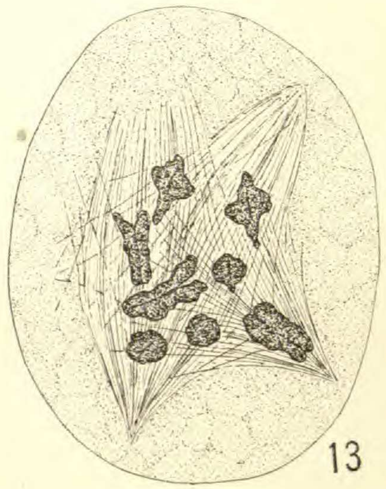
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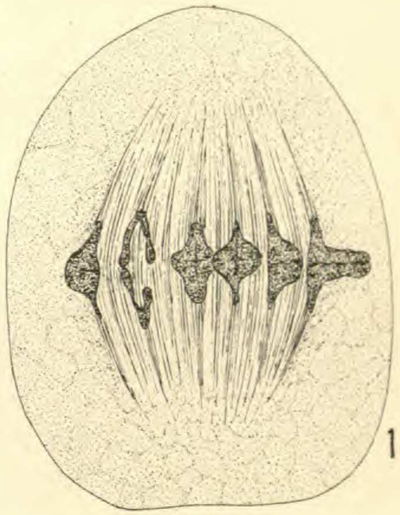
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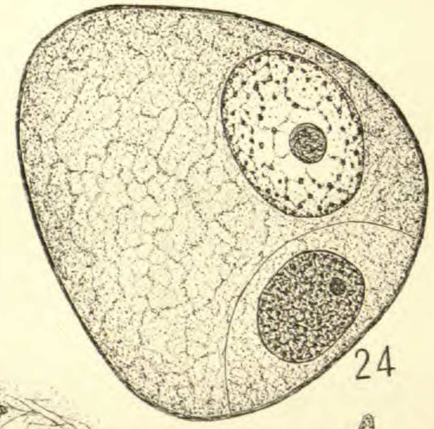
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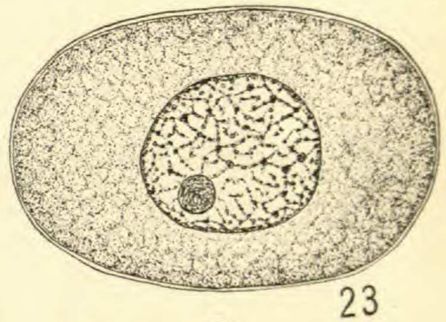
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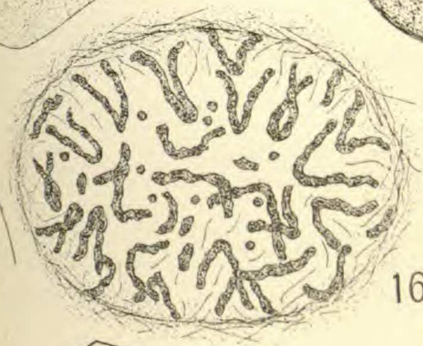
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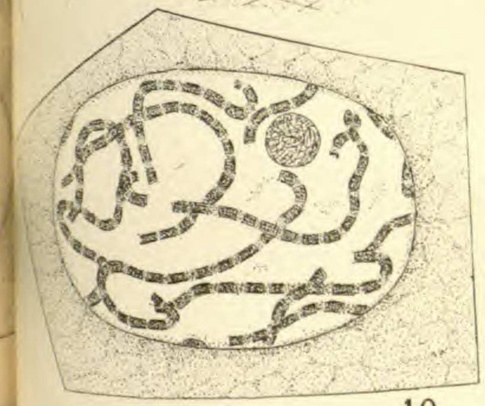
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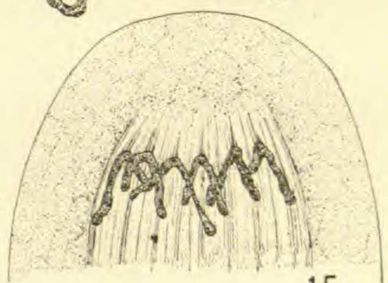
15